New claim 42 has been added along lines of language removed from amended claim 20. New claim 43 finds particular support in Figures 3 and 6.

No new matter has been added by virtue of the amendments.

Turning to the Office Action, claims 32 and 34 were objected to because of informalities. The objections have been addressed by amendment.

Claim 41 stands rejected under obviousness-type double patenting as being unpatentable over claim 9 of U.S Pat. No. 5,747,272. The rejection will be addressed by submission of a Terminal Disclaimer under separate cover.

Claims 1-2, 13-20, 23, 29 and 32-41 stand rejected as being unpatentable under the doctrine of obviousness double patenting over claim 9 of U.S. Patent NO. 5,747,272 in view of Carter (WO 94/04679). Although Applicants respectfully disagree with the stated basis of rejection, it will be addressed by submission of the Terminal Disclaimer.

Claims 1, 2, 13-20, 23, 29 and 32-41 stand rejected under 35 USC §112, first paragraph, as not being enabled for humanized monoclonal antibodies based on antibodies 13C4 or 11E10. Applicants respectfully traverse in part.

Applicants respectfully disagree with the rejection insofar as claims 32-41 already feature particular monoclonal antibodies based on the 13C4 or 11E10 monoclonals. For instance, claim 32 recites specific amino acid sequences derived from the 11E10 antibody. Claims 33-40 are dependent thereon. Claim 41 provides for specific 13C4 and 11E10 antibodies. Accordingly, it is not seen how claims 32-41 can be rejected in view of the position taken by the USPTO. Reconsideration and withdrawal of the rejection as to claims 32-41 are requested.

Applicants have amended claim 1 to recite a more specific humanized monoclonal antibody in which the variable region is based on particular 13C4 or 11E10 sequence. Thus, the claimed antibodies are based on specific antibodies as requested by the Examiner.

In view thereof, reconsideration and withdrawal of the rejection are requested.

Claims 23 and 29 stand rejected under 35 USC §112, first paragraph, on grounds that the specification is not enabled for pharmaceutical compositions other than those comprising humanized monoclonals based on the 13C4 or 11E10 antibodies. Applicants respectfully disagree. However, the rejection has been addressed by this submission. Reconsideration and withdrawal of the rejection are requested.

Claims 13-18, 29, and 32-41 stand rejected under 35 USC §112, second paragraph, on various grounds. Basis for each ground of rejection has been addressed.

Specifically, claims 13, 15 and 16 have been canceled.

The rejection as to claim 20 has been addressed by amendment and addition of new claim 42.

Applicants respectfully disagree with the rejection of claim 32. The phrase "comprises amino acid sequences" would not be viewed as vague and indefinite to one working in this field. The recited variable region would be understood to include all of the sequences in the Markush group in addition to other sequences such as framework sequences.

Claims 1,2, 13-20, 23, 29 and 32-41 stand rejected as being obvious over the Spiers reference, the O'Brien patent, in view of a PCT application by Carter et al. Applicants respectfully disagree with the rejection as follows.

As cited, the Carter reference discloses a method for making a humanized antibody that includes swapping, in the antibody variable region, human and murine CDRs. The reported

result is a humanized antibody that includes a **chimeric (mouse-human) variable region**. See Carter at pg. 5, lines 1-26, for example. In contrast, the humanized antibody of Applicants' amended claim 1 features a **murine** variable region. Applicants' murine variable region is structurally different from the chimeric variable region of Carter. For instance, Carter's chimeric variable region is reported to have one or more "imported" murine CDRs embedded in human variable region sequence. In contrast, Applicants' murine variable region is, by definition, a mouse sequence. The Spiers and O'Brien references, taken individually or together, do not remedy this defect. Moreover, there is no implicit or explicit teaching in the combination of cited references to produce humanized antibody with Applicants' murine variable region sequence.

On this basis alone Applicants respectfully request reconsideration and withdrawal of the rejection.

Applicants disagree with the rejection on further grounds.

For example, and at pg. 9 of the Office Action, the position was taken that:

> Since the amino acid sequence is an inherent property of any protein it would have been *prima facie* obvious for one of skill in the art at the time the invention was made to synthesize and express humanized chimeric antibodies which bind to shiga toxins utilizing the methodology of Carter et al.

Applicants agree with the Examiner that there is almost universal recognition that an amino acid sequence is an inherent property of a protein. But it is certainly not obvious how to obtain that amino acid sequence for the 13C4 or 11E10 murine antibodies from the cited combination of references.

As discussed throughout the present specification, Applicants cloned the 13C4 and 11E10 murine antibodies to learn, among other things, the amino acid sequence. See, for instance, Example 1 on pg. 11 (cloning of 13C4 variable region) and Example 4 on pg. 19 (cloning the 11E10 variable region). Those and related manipulations used to identify the antibody amino acid sequences were needed to humanize the antibodies. See

Example 2 on pg. 13 and Example 5 on pg. 23 (providing for fusion of the murine variable region to human constant region). The cited references, taken alone or in combination, provide absolutely no sequence information about the 13C4 or 11E10 murine antibodies that were cloned and identified by Applicants. It is not seen how those references render the claimed invention obvious.

As understood, the obviousness rejection at pg. 9 of the Action is grounded in the belief that because amino acid sequence is inherent to a protein that the sequence itself must be obvious to identify. But what is inherent is not always obvious to isolate. That is certainly true for the 13C4 and 11E10 antibodies for reasons that follow.

Applicants submit herewith the Declaration of Hing C. Wong under 37 CFR 1.132. According to the Declaration, and for reasons mentioned above and in the prior response, it would not be obvious to identify the amino acid sequence of the 13C4 and 11E10 antibodies using the approach suggested by the USPTO at pg. 9 of the Office Action.

More specifically, at ¶ 8 of the Declaration, Dr. Wong states and confirms that none of the Spiers, O'Brien, Carter and Shitara provide nucleic acid or amino acid sequence information that would be useful to clone the 13C4 or 11E10 murine antibodies.

Moreover, for technical reasons summarized at ¶¶ 9-19 of the Declaration, Dr. Wong states that a worker in this field would be dissuaded from isolating the sequence of the 13C4 and 11E10 murine antibodies using the approach suggested by the USPTO at pg. 9 of the Office Action. In particular, the approach suggested by the Examiner would not work. Declaration at ¶ 9.

According to Dr. Wong, it would be difficult or even impossible to identify V region sequence using the approach urged by the Examiner. Declaration at ¶ 10. More specifically, the significant genetic complexity of the 13C4 and 11E10 murine antibody

variable regions would make using the Examiner's approach to isolate cDNA from a hybridoma library very difficult with no reasonable chance of success. Declaration at ¶11.

Instead, Dr. Wong and his co-inventors developed a particular PCR approach that considered the library complexity and was used successfully to isolate nucleic acid sequence encoding the 13C4 and 11E10 murine antibodies. Declaration at ¶¶ 11-18.

In particular, Dr. Wong states that he avoided the kind of approach urged by the Examiner to isolate cDNA encoding the 13C4 and 11E10V regions. He and his co-inventors designed a news combinations of oligonucleotides ("oligo cocktails") to isolate the 13C4 and 11E10 antibody variable regions. Declaration at ¶¶12 and 16.

For isolation of the 13C4 antibody sequence, Dr. Wong stated that he developed 13 distinct degenerate oligonucleotide primers. Declaration at ¶ 13. In view of the degeneracy, these provided **359 discreet sequences** for isolating the 13C4 heavy chain and **200 discreet sequences** for isolating the corresponding light chain. Declaration at ¶ 14. When viewed as primer pairs, Dr. Wong stated that for successful PCR amplification, the equivalent of **2, 118 possible pairs of primers** were used to isolate the 13C4 heavy chain and **4,224 possible primer pairs** were used to isolate the corresponding light chain. Declaration at ¶ 15.

To isolate the sequence of the 11E10 antibody, Dr. Wong stated that he developed six light chain primers and eight heavy chain primers. Declaration at ¶16. These represent 16 distinct degenerate oligonucleotide primers. These provided **544 discreet sequences** for isolating the heavy chain when degeneracy was considered. Declaration at ¶ 17. In terms of primer pairs, Dr. Wong stated that to achieve amplification, he and his co-inventors had to use the equivalent of **67,584 possible pairs of primers to isolate the heavy chain of 11E10** and **4,224 possible primer pairs for the light chain.** Declaration at ¶18.

None of the discreet primer sequences or substantial number of possible primer pair combinations Dr. Wong and his co-inventors found were needed to isolate the 13C4 and11E10 antibody sequences are taught or even suggested by the cited references.

Dr. Wong and his co-inventors identified and understood the problems inherent in the Examiner's suggested cloning approach. They solved that problem by recognizing a need for balance between two competing PCR factors: 1) complexity of their oligo cocktail and 2) V region PCR amplification efficiency. Declaration at ¶ 19. Dr. Wong and his co-inventors discovered that by amplifying 80% of the V region genes in the library, that level of amplification provided the needed balance between the competing factors. Specifically, about 500 pmoles of primer per reaction provided the required amplification and led to isolation of the antibody sequences.

Neither the PCR amplification problem identified and understood by Dr. Wong and his colleagues or its solution is taught or suggested by the cited references taken alone or together.

In view thereof, there is no basis for maintaining the instant obviousness rejection. Reconsideration and withdrawal therefore are requested.

Claims 1, 2, 13-20, 23, 29 and 32-41 stand rejected as being unpatentable over Speirs or O'Brien in view of Shitara et al. (U.S Pat. No. 5,866,692). Applicants respectfully traverse for reasons mentioned above and in the prior response.

Specifically, Dr. Wong stated that the Examiner's combination of references did not obviate the present invention and one would be dissuaded from using the suggested cloning approach to obtain sequence for the 13C4 and 11E10 antibodies. Declaration at ¶¶7-8. The suggested approach would not work. Declaration at ¶ 9. The PCR amplification approach

worked out by Dr. Wong is not taught or suggested by the references as relied on. See the discussion above and Declaration at ¶ 10-19.

Although it is not believed that any additional fee is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. **04-1105** should any fee be deemed necessary.

Attached to this submission is a marked-up version of the changes made to the specification and claims. The attached page is captioned "version with markings to show changes made".

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 13, 15, and 16 were cancelled.

The following claims were amended.

1. (Amended) A humanized monoclonal antibody that binds to Shiga toxin protein, comprising a constant region and a murine variable region, wherein said constant region contains at least part of a human immunoglobulin constant region and said murine variable region contains at least part of a [nonhuman] murine immunoglobulin variable region as shown in Figure 3 (SEQ ID NO: 21) or Figure 6 (SEQ ID NO: 42), wherein the antibody specifically reacts with Stx1 or Stx2 antigen.

32. (Amended) A [hmanized] humanized monoclonal antibody that binds to a Shiga toxin protein comprising a human immunoglobulin constant region and a variable region,

wherein the variable region comprises amino acid sequences selected from the group consisting of amino acids 31-35 of SEQ ID NO: 44, amino acids 50-66 of SEQ ID NO: 44, amino acids 99-108 of SEQ ID NO: 44, amino acids 24-40 of SEQ ID NO: 42, amino acids 56-62 of SEQ ID NO: 42, and amino acids 95-103 of SEQ ID NO:42.

33. (Amended) A fragment of the antibody of claim [30] 32 wherein the fragment binds a Shiga toxin protein.

34. (Amended) [A] The humanized monoclonal antibody of claim [30] 32 wherein the human constant region is selected from the group consisting of IgG, IgA and IgM.

35. (Amended) The humanized monoclonal antibody of claim [30] 32 wherein the human constant region is IgG.

36. (Amended) The humanized monoclonal antibody of claim [30] 32 wherein the human constant region is IgG1-kappa.

37. (Amended) The humanized monoclonal antibody of claim [30] 32 wherein the variable region comprises the amino acid sequence of SEQ ID NO: 44.

38. (Amended) The humanized monoclonal antibody of claim [30] 32 wherein the variable region comprises he amino acid sequence of SEQ ID NO: 42.

39. (Amended) A pharmaceutical composition comprising an antibody of claim [30] 32 and a pharmaceutically acceptable carrier or diluent.

40. (Amended) A pharmaceutical composition comprising an antibody fragment of claim [31] 32 and a pharmaceutically acceptable carrier or diluent.

The following new claims 42-43 were added.

42. (New) The humanized monoclonal antibody of claim 20, wherein the variable region comprises amino acid sequences selected from the group consisting of amino acids 31-35 of SEQ ID NO: 44, amino acids 50-66 of SEQ ID NO: 44, amino acids 99-108 of SEQ ID NO: 44, amino acids 24-40 of SEQ ID NO: 42, amino acids 56-62 of SEQ ID NO: 42, and amino acids 95-103 of SEQ ID NO:42.

43. (New) The humanized monoclonal antibody of claim 1, wherein the human immunoglobulin constant region is as shown in Figure 3 (SEQ ID NO: 19) or Figure 6 (SEQ ID NO: 44).